REVIEW ARTICLE

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Complex Effects of Melatonin: the Ubiquitously Acting Pineal Hormone

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A few decades ago, melatonin was linked to phenomena such as sleep and circadian rhythms. Of late, the functions of melatonin include effects on the immune system, as an anticancer and oncostatic agent, as a free radical scavenger, as an antioxidant, an antiinflammatory agent, a sleep promoting agent and an agent to improve mental performance. Novel chronobiotic and relevant properties of melatonin have been reported. This review focuses on the complex effects of melatonin and its ubiquitous actions in mammals and humans.

Circadian rhythms are endogenously generated which are exhibited as approximately 24-hour oscillations in hormones, sleep state, alertness, performance and other physiologic functions. These rhythms are generated by a pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus[1]. Since the endogenous rhythm is not exactly 24 hours in length, the pacemaker needs to be periodically reset to local environmental time, so that the pacemaker and the resulting circadian rhythms remain entrained to local time.

One of the sites of melatonin synthesis, the pineal gland, was thought to be totally without function until 40 years ago. It now appears that its major secretory product, melatonin, may influence the physiology of many tissues in the organism[2]. Recent studies have shown that melatonin is phylogenetically a very old molecule and possibly exists in all animals, from algae to humans. Melatonin is estimated to have evolved 2.5-3.0 billion years ago, coincident with the development of oxygen-based metabolism. During evolution, melatonin is claimed to have mediated dark adaptation and thus the daily night pulse or melatonin may have been used by organisms in transduction of the photoperiodic message, a key temporal cue for almost all early life forms[3].

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The pineal gland and melatonin were initially identified as the interface between the prevailing environmental photoperiod and seasonal reproductive capability in photoperiodic mammals. Subsequently, melatonin was linked to phenomena such as sleep and circadian rhythms. Further, functions of melatonin include effects on the immune system and as an anticancer and oncostatic agent[4,5]. These effects could also be due to antioxidant properties of melatonin.

Rhythmic production of melatonin:

Levels of melatonin are low during day and high during night (10-20 times) in mammals and humans. Many lines of evidences indicate that the SCN of the hypothalamus in mammals generate circadian rhythms including that of pineal melatonin secretion[2]. At sunset, the cessation of light triggers neural signals, which stimulate the pineal gland to begin releasing melatonin. This rise continues for hours, eventually peaking around 2 a.m. (3 a.m. for the elderly) after which it steadily declines to minimal levels by morning. Melatonin production normally peaks around the time of puberty and then decreases with age. The most rapid decline usually occurs after age 40[6]. Large inter individual differences exist in the amount of melatonin excreted; however, within an individual differences exist in the amount of melatonin excreted; however, the profile is relatively stable across daily cycles[7]. The melatonin profile is not affected by the gender of the subject, or, in women, the phase of the menstrual cycle[8]: In mammals, the night time production of melatonin

is mainly driven by the circadian clock (SCN), which controls the release of norepinephrine from the dense pineal sympathetic afferents. Besides the noradrenergic input, the presence of numerous other transmitters (neuropeptides) originating from various source has been reported to regulate melatonin synthesis by modulating the effects of norepinephrine in the pineal gland[9].

Retinal rhythmic production of melatonin:

Melatonin is rhythmically produced in the retina in all the species so far investigated; melatonin is high during the night and low in the day[10]. Exposure to light at night rapidly depresses pineal melatonin levels and subsequently blood melatonin levels drop precipitously[10,11]. In non-mammalian vertebrates retinal melatonin can enter the blood circulation, and thus contribute to the circulating level, whereas in mammals the available evidences suggest that retinal melatonin does not contribute to circulating melatonin levels. The finding that retinal melatonin in mammals does not enter the circulation suggests that its action must be within the retina[10].

The classical photoreceptor cells, i.e. the rods and cones were not to be involved in the pineal melatonin production. Rather, there are some specialized neurons which contain a unique photopigment in the retina that respond to light. This information is transduced into neural message to the hypothalamus through retinohypothalamic tract[12].

Melatonin receptors:

The SCN of the hypothalamus have melatonin receptors and melatonin may have a direct action on SCN to influence circadian rhythms[13]. Melatonin is metabolized to 6-hydroxy melatonin in the liver and the main metabolite excreted is 6-sulphatoxy melatonin[14].

Membrane melatonin receptors have transmembrane domains and they are in the super family of G-protein-coupled receptors. Two receptor sub types (MT_1 and MT_2) with high affinity for melatonin have been cloned and characterized in a variety of human tissues including SCN, but it seems that few tissues are devoid of melatonin receptor[15]. There are a number of conditions which are said to be improved by treatment with melatonin – which are mediated by melatonin receptors[14,16] such as tinnitus[17], facilitating the termination of benzodiazepine treatment while maintaining good sleep quality[18] and cancer[19]. However, the role of melatonin receptor in day-to-day actions remains to be assessed.

Chronobiological and relevant properties of melatonin:

Melatonin (i) shifts circadian rhythms, (ii) can entrain free running sleep-wake cycles to shorten the periodicity of sleep-wake cycles, (iii) induces transient sleepiness or lowered alertness, (iv) can reduce sleep latency, increase sleep efficiency and affect the EEG and (v) can partially counter conflicting light treatment when adapting to phase shift[20].

Melatonin as a biological rhythm regulator:

Melatonin has been found to alter the timing of mammalian circadian rhythms and has been shown to function in concert with light to hold the circadian rhythms in tune with prevailing environmental light-dark cycle. This has prompted many investigators to label this hormone as a circadian chemical messenger or chronobiotic[21]. The fact that melatonin is secreted in a circadian manner and this rhythm is governed by SCN and modified by light-dark cycle suggested that this hormone is concerned with regulation of circadian rhythmicity. Moreover, melatonin has been termed as an internal *zeitgeber* regulating rhythms occurring in every cell, organ and tissues present in human body[22].

The primary function of melatonin in mammals is to convey information about the changing length of night in the course of the year. Melatonin reinforces functions associated with darkness[23]. The recent explosion of interest in melatonin as a therapeutic agent in conditions ranging from cancer to jet lag had thrown light on investigations in terms of understanding its mode of action. The rhythm of melatonin synthesis in pineal gland in mammals and humans is generated in the suprachiasmatic nuclei (SCN) and synchronized to the 24-hour day primarily by the light-dark cycle. Removal of pineal gland abolishes the rhythm and leads to low or undetectable levels in plasma[24]. In all living beings, from the simplest organisms to humans, circadian rhythms have long been organized as important coordinators of various physiological functions. These rhythms may influence the evolution of several pathologies, including stroke[25]. Furthermore, melatonin deficiency develops normally during aging, and aging has been shown to worsen the outcome of experimental stroke[25].

Melatonin as an antioxidant:

The ability of melatonin to neutralize the $^{\cdot}OH$ was almost a serendipitous discovery and followed a hypothesis that some of melatonin's effects were receptor independent. In 1993, all of melatonin's known actions were believed to involve well-characterized and subsequently cloned, cellular membrane receptors. The ability of melatonin to directly

scavenge the ·OH was proven using a combination of spin-trapping methodologies and electron spin resonance (ESR) spectroscopy[36].

Melatonin also reportedly neutralizes hydrogen peroxides (H_2O_2) and other oxidants including singlet oxygen ($^1O_2$), nitric oxide (NO·) and the product of the interaction of the superoxide anion radical ($O_2^{\cdot-}$) namely, peroxynitrite anion (ONOO·) and /or its metabolite[27].

Free radical damage has been implicated in a wide variety of diseases has been implicated in a wide variety of diseases and melatonin has been shown to be protective including ischemia/reperfusion injury (in the brain, gut, liver, lung, etc.), toxic drug exposure, bacterial toxin exposure, heavy metal toxicity, Schistosomias, Alzheimer's diseases, Parkinsonism, skin erythema and tardive dyskinesia[15].

Melatonin, in pharmacological concentrations, has the capability of increasing either mRNA levels or the activites of major antioxidative enzymes. There is also evidence that the physiological nighttime increases in melatonin are associated with a rise in the activities of these key antioxidative catalysts. In addition, surgical removal of the pineal gland, one source of melatonin in vertebrates, lowers circulating melatonin levels and exaggerates oxidative damage, attesting to the relevance of melatonin as a physiological antioxidant[28].

Melatonin and intestinal motility:

Melatonin binding sites have been identified in the gut. Despite few studies, the physiological role of melatonin in gut function remains unclear. Studies by Merie *et al.*[29] indicated that, endogenous melatonin is physiologically involved in the pre-and postprandial changes of intestinal motility at night. Furthermore, exogenous melatonin produces pharmacological effect on pre and postprandial intestinal motility.

Melatonin and antiinflammatory effects:

The antiinflammatory effects of melatonin depend on the combination of the following pharmacological properties: Melatonin secondarily scavenges and inactivates $O_2^{\cdot-}$, which reduced the formation of $ONOO^-$. This, in turn, prevents the activation of poly (ADP ribose) synthase and the associated tissue injury. At the same time, melatonin lowers the synthesis of NO, thereby also reducing $ONOO^-$ formation. Finally, melatonin reduces the recruitment of polymorphonucleases into the inflammatory site[16,5]. This effect of melatonin is very likely secondary to the reduction in endothelial oxidant injury, and, hence, a preservation of endothelial function[30]. These findings support the view that the over-production of reactive oxygen species contributes to the acute inflammatory response and small molecule such as melatonin which permeated biological membranes and functions as intracellular radical scavenger, may be useful in the therapy of conditions associated with local or systemic inflammation[31].

Melatonin and immune system:

A number of publications in experimental animals and human individuals on this subject, are extensive and the findings were summarized in several reviews[15,32]. Enhanced immune function in short days is correlated with increased duration of nightly melatonin secretion and recent studies indicate that melatonin can act directly on immune cells to enhance immune function[16]. It remains unknown, which melatonin subtype mediates immune enhancement by melatonin[16].

Melatonin has also been shown to improve immunity and extend lifespan in rodents. Nighttime administration of melatonin can also counteract the immune-suppressing effects of acute anxiety stress in mice.

In human beings, oral administration increases natural killer (NK) cell activity[15]. Melatonin was reported to regulate gene expression of several immunomodulatory cytokines including tumor necrosis factor-α (TNF-α), transforming growth factor-α (TGF-α) and stem cell factor (SCF) and as well as the levels of interleukin-1[32]. Finally, melatonin is a potent inhibitor of apoptosis in immune cells[15].

Melatonin and cancer:

The loss of pineal function and the resulting decreased melatonin serum levels in night shift workers may increase reproductive hormone levels and in particular, estradiol levels, there by stimulating the growth of hormone sensitive tumors in the breast[33]. Today, many of oncostatic properties of melatonin have been well described[34] and evidence from experimental studies strongly suggests a link between tumor suppression and melatonin[35]. *In vitro* studies, give support to reduction in the growth of malignant cells of the breast, and other sites by both pharmacological and physiologic doses of melatonin[36]. Furthermore, melatonin also appears to inhibit tumour growth and is most effective when given in the evening[4]. Melatonin may augment the anti tumour activity of IL-2 (interleukin 2) by inhibiting tumour growth factor production[4].

In rodents, pinealectomy boosts tumor growth, whereas exogenous melatonin administration exerts anti-initiating[37]

and oncostatic activity in various chemically induced cancers[35]. Reports show that melatonin exhibits a growth-inhibitory effect on endometrial, ovarian carcinoma cell lines, Lewis lung carcinoma, prostate tumor cells and intestinal tumors[36].

Melatonin and aging:

Recent studies indicate that decreased binding of melatonin to SCN in old rats is correlated with disruption in overt circadian rhythmicity[38]. Further support for linkage between melatonin and aging is the finding that increased longevity in rats after life-long food restriction is associated with improved pineal function and increased melatonin levels[39]. In a few studies using wrist activity as a marker of sustained sleep in elderly subjects complaining of insomnia, both low and high doses of melatonin were found to reduce motor activity at night[40].

The potential use of melatonin replacement for treating sleep-wake disorders in elderly is a particularly attractive hypothesis since; (i) disturbed sleep becomes more prevalent with advanced age[41] (ii) melatonin levels decline with age[38] and (iii) melatonin levels are reported to be significantly lower in elderly insomniac patients than in age-matched controls[38].

Free radicals are believed to be the culprits in some of the degenerative physiological changes associated with aging. Melatonin is the only antioxidant known to wane with age. Thus, after middle age, circulating levels of melatonin gradually diminish in all species, which contrasts with other antioxidants. This age-related reduction in melatonin is correlated with parallel depression in the total antioxidative capacity of human serum[42].

In humans, melatonin concentrations exhibit a clear circadian rhythm around the 6th month of life and reach the highest levels between 4 and 7th year age. At puberty, there is a drop in melatonin concentrations and thereafter its plasma concentrations diminish gradually[43]. There are number of possible reasons for the age related melatonin production[44] including a) clock related disturbances, b) abnormalities in entrainment, c) insufficient *zeitgebers* and d) pineal gland dysfunction.

MELATONIN AND PSYCHIATRIC DISORDERS

Insomnia:

It has been suggested that melatonin improves sleep functioning, and this possibility has been recently tested in medical populations and it was found out that melatonin may be a useful hypnotic for medically ill patients with initial insomnia, particularly those for whom conventional hypnotic drug therapy may be problematic[45]. Several mechanisms have been suggested that could mediate the sleep promoting effects of this hormone. These include the correction of circadian deregulation, the attenuation of the daytime alerting process generated by the suprachiasmatic nucleus, and the lowering of core body temperature[45]. Melatonin, by correcting circadian rhythms, should, theoretically, improve mental performance.

Alzhemier's disease:

Recent studies have suggested that melatonin in the cerebrospinal fluid of patients with Alzhemier's disease compared to age-matched control subjects. Since circadian rhythms are disrupted in Alzhemier's disease, it is interesting to speculate whether restoration of melatonin to normal levels in these patients would alleviate other symptoms as well[46].

Hypernychthemeral syndrome:

Hypernychthermeral syndrome is characterized by intermittent insomnia that recurs with a regular periodicity over several days. It represents the failure of the circadian clock to entrain normally to the environmental light-dark cycles[47]. Rhythmic administration of melatonin appears to allow stable entrainment of the circadian oscillator of these patients to the desired schedule.

Winter depression and schizophrenia:

The psychiatric diseases like winter depression and schizophrenia result in decline in amplitude of melatonin rhythm[48]. There is also a claim, though controversial, of delayed melatonin rhythm in patients with seasonal affective disorder (SAD). Melatonin by improving the quality of sleep in the night can indirectly help to better mood and enhance performance. Significantly, antidepressant drugs cause increase in melatonin secretion. All these raise the possibility for a close link between melatonin production and psychiatric disorders[48].

CONCLUSIONS

The majority of published literature discussed in this review indicates that melatonin has therapeutic benefits in circadian rhythm related sleep disorders. However, yet its mechanism of action remains unclear and little information is available on its important function as a photoneuroendocrine transducer in humans. Much further research is needed on its physiological role and pharmacological effects in humans.

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